

The Experimental Effects of Lipopolysaccharide-Induced Renal Injury in Mice Model

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ABSTRACT

Acute renal injury (AKI) is a serious case with a high mortality rate, especially among hospitalized patients. It could be induced by sepsis, hypovolemia, atherosclerosis, and ischemia. Sepsis is caused mainly by Lipopolysaccharides (LPS) of gram-negative bacteria. The current work aimed to assess the potential ability of LPS to induce AKI in mice as well as the probable mechanism. Experimentally, three different doses of LPS (2.5, 5, and 7.5 mL/kg) were evaluated. Results showed that one-time LPS injection in dose (2.5 mg/kg, i.p.) was optimum to induce AKI in mice. Moreover, LPS induced severe pathological injuries in kidney tissues. It significantly increased the blood urea nitrogen and serum creatinine in samples of LPS-exposed mice, demonstrating deteriorated kidney function. Notably, LPS increased tumor necrosis factor (TNF)- α in renal tissue homogenate, indicating its ability to initiate a severe inflammatory cascade. In summary, LPS (2.5 mg/kg, i.p, single dose) has a potential capability to induce AKI with inflammatory insult.

Keywords: Acute kidney injury; lipopolysaccharides; TNF- α ; inflammation; BUN.

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1. INTRODUCTION

Acute kidney injury (AKI) is a critical case with a high rate of morbidity and mortality in patients [1]. Epidemiologic investigation showed that AKI in hospitalized patients developed in over half of all intensive care unit patients [2]. AKI has a relatively high mortality rate which is about 30% [3]. There are many causes of AKI such as sepsis, circulatory shock, and medication side effects [4].

Sepsis is one of the main causes of AKI and septic AKI is more severe than non-septic AKI [5]. Many types of infection can lead eventually

to systemic inflammation and so sepsis, and this is the most probable cause of AKI [6]. Sepsis is mainly caused by gram-negative bacterial endotoxins which are released into circulation and activate inflammatory cascades in the kidney [7]. Resulting in excessive production of inflammatory mediators and cytokines [8]. LPS is a component of gram-negative bacteria wall and it has a virulent effect on many organs including kidneys. LPS-induced kidney injury is characterized by renal tissues inflammation, and tubular dysfunction [9].

To manage the septic effect on renal injury in humans, we must have a similar model on mice

with the same pathological effects on renal tissues. Therefore, the current study aimed to assess the potential ability of LPS to induce AKI in mice as well as the probable mechanism

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

LPS (from *E. coli* 0111:B4) was purchased from Merck (St. Louis, MO, USA). LPS was dissolved in Phosphate buffered saline (PBS) and administrated i.p. Blood Urea Nitrogen (BUN) and Serum Creatinine (SCr) kits were obtained from Greiner Diagnostic GmbH, Germany. TNF- α ELISA kit was purchased from CUSABIO, China. Other used reagents and solvents were of the highest analytical grade available.

2.2. Animals

BALB/c male mice (4–6 weeks old) were obtained from the Nile Co. for Pharmaceutical and Chemical industries, Cairo, Egypt. Ad libitum access to water and food was provided to mice for seven days before the experiment in a temperature and humidity-controlled room. Experimental work had approval no. : 114 from Ethical Committee for the use of animal subjects, Faculty of Pharmacy Ain Shams University, Cairo, Egypt.

2.3. Experimental Model

Mice were divided randomly into four groups (eight animals each). The first group named as control group received the vehicle only (PBS) by i.p. injection. The second, third, and fourth groups received single i.p injections of LPS at doses (2.5, 5, 7.5 mg/kg), respectively. Collection of blood samples was done after 24 h, samples than allowed to clot and separated using centrifugation method to collect sera. After that, animals were sacrificed for separating kidney tissue samples. Finally, kidney samples were dissected, washed with saline, and kept at -80 °C till homogenization. To perform the

histopathological examination. Representative renal tissue specimen from each group was fixed in 10% formalin/saline for 24 h.

2.4. Histopathology Examination

Formalin-fixed kidney samples were processed and embedded in paraffin and cut at 4 μ m thickness. Then, deparaffinization of tissue samples was done followed by staining on glass slides by hematoxylin and eosin (H & E). Finally using a light microscope, the pathological changes in renal tissues were detected.

2.5. Blood Urea Nitrogen and Serum Creatinine levels determination.

Serum samples were used for detection of BUN and creatinine levels in different groups by auto analyzer using commercially available kits as stated by manufacturer's instructions.

2.6. TNF- α assay

TNF- α levels in renal tissue homogenates were assessed by a specific ELISA kit (CUSABIO, China) based on the sandwich technique, according to the manufacturer's instructions.

2.7. Statistical analysis

Data are presented as mean \pm standard deviation (SD). A one-way ANOVA test was used to evaluate differences between groups. All statistical analyses were performed, while the graphs were drawn using a prism computer program (Graph Pad Software Inc., La Jolla, CA, USA) with $P < 0.05$ means statistically significant.

3. RESULTS

3.1. Histopathological examination

For the highest dose (7.5 mg/kg), the mortality rate exceeded 70% of the group, so it was neglected (**Fig. 1D**). Consequently, the lower doses (2.5 and 5 mg/kg) were evaluated relative to the control group. Histopathological changes

in kidney tissues were assessed 24 h after LPS treatment, as shown in **Fig. 1**. The control group (1A) showed normal kidney histological structures. On contrary, LPS-exposed animals at both doses (2.5 and 5 mg/kg) showed histopathological changes in a dose-related

manner, as displayed in **Fig. 1B & 1C**. These were manifested by congestion of blood vessels associated with perivascular edema as well as focal hemorrhage at the cortex. Also, degenerative change was detected in the lining epithelium of the tubules.

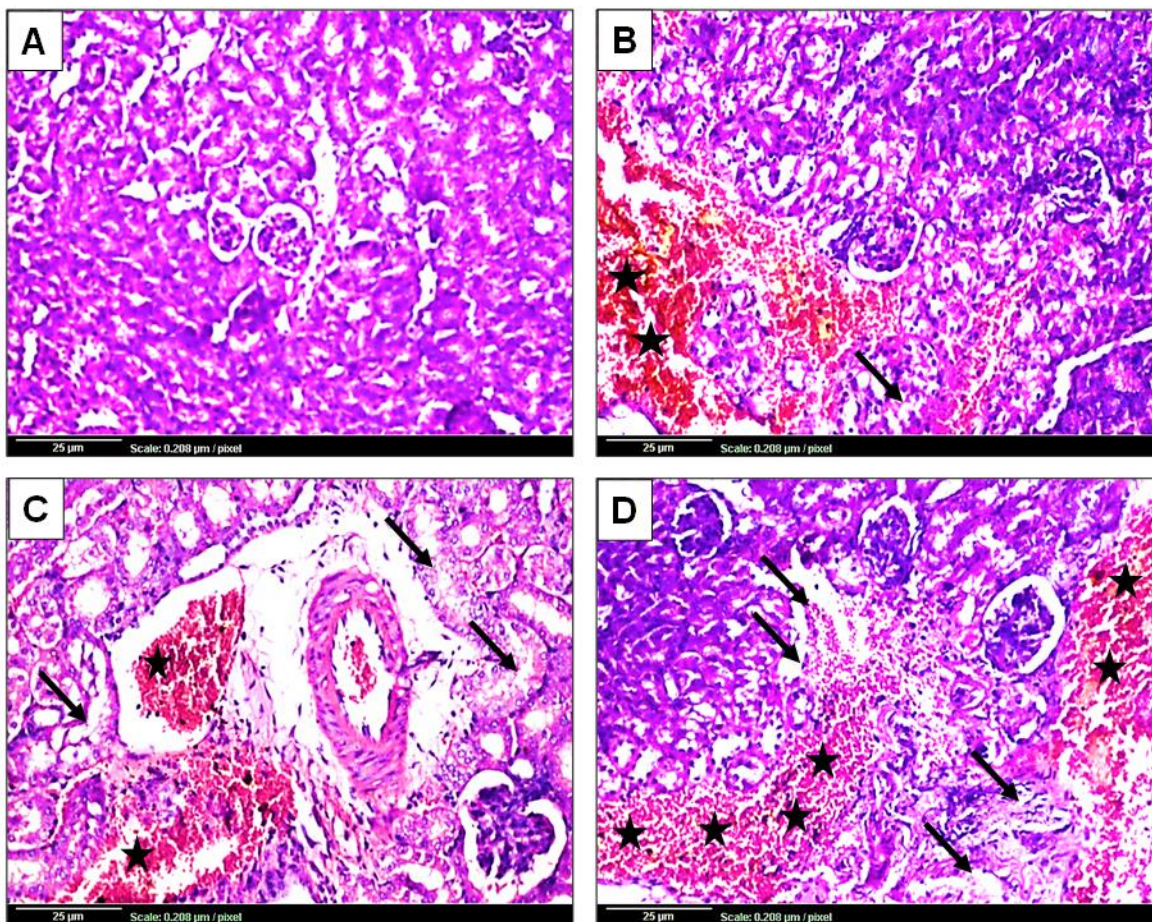


Fig. 1. Representative photomicrographs of mice kidney sections stained by Hematoxylin & Eosin (H & E; x100) after 24 h exposure to Lipopolysaccharide (LPS) single dose. A) Control group with normal renal architecture; B) LPS, 2.5 mg/kg; C) LPS, 5 mg/kg; D) LPS, 7.5 g/kg. There are dose-related changes as manifested by congestion of blood vessels and perivascular edema (stars) with degenerative changes in the tubular lining epithelium (arrows)

3.2. Effect of LPS on BUN and serum creatinine

Renal functions were evaluated by both BUN and serum creatinine levels. As shown in **Fig. 2 & 3**, the levels of BUN and serum creatinine were elevated in a significant way in comparison to the control group. It is worthy to note that no significant difference was detected between LPS

groups (2.5 & 5 mg/kg).

3.3. Effect of LPS on TNF- α concentration in renal tissue homogenate

The cytokine TNF- α and total proteins were assessed in renal tissue homogenate, and results were standardized per sample protein content in renal homogenate samples as previously described [10]. Notably, it could be inferred that

LPS increased inflammatory reactions in renal tissue, as indicated by a significant rise of TNF- α concentration compared to control, as shown in Fig. 4.

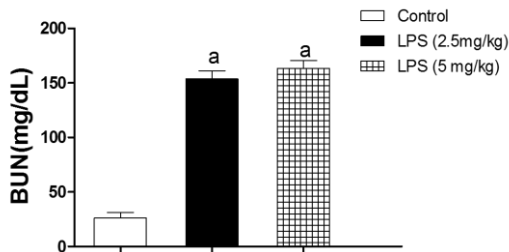


Fig. 2. Effect of LPS doses (2.5 mg/kg, 5mg/kg) on BUN levels in mice model. The values presented are the mean \pm SEM (n= 8 in each group). a<0.01 compared to the Control group, P<0.05. One way ANOVA test was used to analyze data

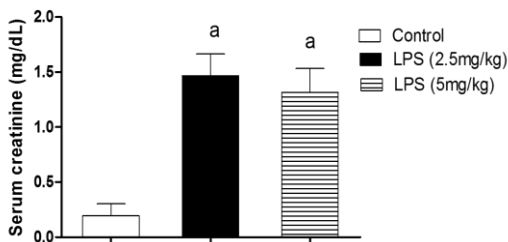


Fig.3. Effect of LPS doses (2.5 mg/kg, 5mg/kg) on serum creatinine levels in mice model. The values presented are the mean \pm SEM (n=8 in each group). a<0.01 compared to the Control group, P<0.05. One way ANOVA was used to analyze data

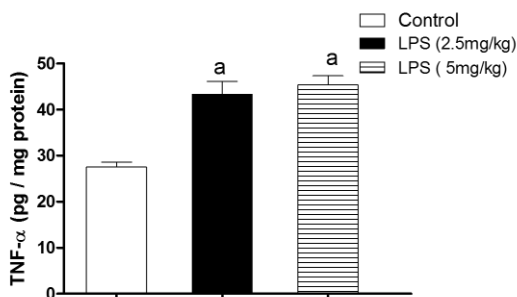


Fig.4. Effect of LPS on TNF- α production in kidney tissues. The values presented are the mean \pm SEM (n= 8 in each group). a<0.01 compared to the Control group, P<0.05. One way ANOVA was used to analyze data.

4. DISCUSSION

Acute renal injury is a serious clinical problem characterized by a rapid decline in function of the kidneys causing a disturbance in the body fluid homeostasis [7]. AKI is associated with sudden tubular and endothelial cell injury in kidney tissues then leading to complicated effects on other organs and systems function and may cause multi-organ failure in patients [11]. Sepsis-induced AKI results in renal function loss and even multi-organ damage leading to a high impact on patients' morbidity and mortality [12]. The pathophysiology of septic AKI is different from non-septic AKI Sepsis is mainly caused by gram-negative bacteria LPS and is characterized as an intense inflammatory response [13]. Therefore, the current study aimed to investigate and assess the potential ability of LPS to induce AKI in mice as well as the probable mechanism.

Histopathological examination of the kidneys of LPS challenged mice showed increased glomerular degeneration (edema) and congestion of blood vessels. Also, focal hemorrhage indicating acute damage in renal tissues can be easily recognized after LPS treatment of mice. BUN and serum creatinine are the main indicators of how the kidney functions are doing [14]. And as AKI is characterized by the sudden loss of kidney function, mice treated with LPS showed significantly high levels of BUN and serum creatinine.

Previous studies demonstrated that high levels of inflammatory cytokines such as TNF- α could increase renal vasoconstriction which in return decreases renal blood flow and so lowering glomerular filtration rate [15]. Consequently, the effect of LPS on initiating inflammatory processes in the kidney was examined by measuring renal TNF- α concentration using the ELISA technique. LPS showed a dramatic increase of TNF- α . This indicates its ability to initiate a severe inflammatory response. TNF- α

and other related pro-inflammatory factors as IL-1 β , and IL-6 promote the production of neutrophils, macrophages, and dendritic cells. Activation of immune cells increases reactive oxidative species (ROS), neutrophils, leading to damage in surrounding tissue [16, 17]. So it is clear that LPS can initiate an inflammatory cascade in renal tissues resulting in AKI [18].

In conclusion, exposure of mice to LPS at a dose of 2.5mg/kg was optimum to induce AKI, which could be justified – at least partly – by the ability to induce a severe inflammatory response.

Declarations

Ethics approval and consent to participate

Not applicable

Consent to publish

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article in the main manuscript.

Competing interests

No competing interests were declared by the authors.

Funding statement

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